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MULTIPLICATION OF THE NEUROTROPIC STRAIN OF RIFT VALLEY  
FEVER IN THE MOUSE SPLEEN AND LIVER

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Since MacKenzie and Findlay<sup>1</sup> used the inhibitory effect of immune serum to produce a neutropic strain of Rift Valley fever virus (RVF) in the mouse, a highly sensitive animal, several investigators succeeded in obtaining similar strains

<sup>2-5</sup>  
by various methods. The strains thus obtained scarcely differ from one another in pathogenicity. Virus inoculated intracerebrally proliferates in the brain to provoke meningoencephalitis. The adult mouse generally survives intraperitoneal injection of the strain. If the dose is high or the mouse inoculated is young, the virus can kill the animal with nervous symptoms. For several days after intraperitoneal injection<sup>the</sup>

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1. R. D. MacKenzie and G. M. Findlay, Lancet, 1936, Vol. 1, p. 140.
  2. Y. Nakamura and Y. Nagano, Compt. rend. XIII<sup>e</sup> Congrès Microbiol. Japon (Proceedings of the 13th Congress of Microbiologists in Japan), 1939, p. 226 (in Japanese).
  3. K. C. Smithburn, Brit. J. Exp. Path., 1949, Vol. 30, p. 1.
  4. S. F. Kitchen, Ann. Trop. Med. Parasit., 1950, Vol. 44, p. 132.
  5. M. Endo, Virus, 1951, Vol. 1, p. 42.

the virus is present in the circulating blood, after which it tends to concentrate in the spleen, a fact ~~which~~ suggests that the neurotropic virus retains some affinity for the viscera<sup>1,6</sup>.

To obtain new data on the behavior of the neurotropic strain in mice, we performed the experiments whose results will be set forth below.

Material and procedure. A neurotropic strain of RVP was used. This strain was obtained by Endo by passaging the pantropic virus in a chick embryonal brain tissue culture. The strain was preserved by passages in the mouse brain. Broth with a slightly alkaline reaction was used to dilute the virulent materials. The latter were titrated by intracerebral inoculation of mice.<sup>5</sup> The LD<sub>50</sub> was calculated by Kaerber's method. The neutralizing power of the serum was evaluated by intraperitoneal injection<sup>7</sup> of mice with 0.1 ml of mixtures of decimal dilutions

6. R. D. MacKenzie, G. M. Findlay, and R. O. Stern, Brit. J. Exp. Path., 1936, Vol. 16, p. 352.
7. G. Kaerber, Arch. Exp. Path. Pharm., 1931, Vol. 162, p. 480.

of the pantropic virus and an equal volume of the serum under study heated to 56° C for 30 minutes. The mixtures of virus and serum were incubated at 37° C for 30 minutes before injection.

The neutralization index was calculated in the routine way.

Distribution of virus at different intervals after intraperitoneal inoculation of mice. We injected mice <sup>intraperitoneally</sup> with 0.2 m of the virus.

The dose was equal to 10<sup>5.17</sup> and 10<sup>8.17</sup> times the LD<sub>50</sub> for

experiments 1 and 2, respectively. The mice were exsanguinated

at intervals and ~~the~~ serum, liver, lung, kidney, spleen, and <sup>(taken)</sup>

brain were removed. The material ~~obtained~~ from 4 mice ~~was~~ was

injected into 4 mice intracerebrally. The results can be

seen in Fig. 1.

The results coincided on essential points with those of  
MacKenzie et al.  
<sup>6</sup>

Virus was detected in the blood, lung, and kidney for 3 or 4 days and in the liver for 5 to 8 days. Virus in fairly large amounts was found in the spleen on days 5, 14, and 21.

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Fig. 1. Distribution of the neurotropic virus of RVF and the neutralizing power of the blood after peritoneal inoculation of mice

- 1 - Blood
  - 2 - Liver
  - 3 - Lung
  - 4 - Kidney
  - 5 - Spleen
  - 6 - Brain
  - 7 - hour
  - 8 - day(s)
  - 9 - Neutralization index
  - 10 - One mouse dead of infection
  - 11 - Intercurrent death
  - 12 - Survival
- 

Virus was present in the brain in very small quantities on day 1 or 2. It could have come from the residual blood. At more advanced stages when it was no longer found in the circulating blood, it appeared in the brain in remarkably large amounts.

Multiplication of virus in the liver and spleen. In order to determine whether the neurotropic strain multiplies in the liver or spleen, we titrated the ~~infectivity~~ <sup>infectiousness</sup> of the blood, liver, and spleen at various intervals after peritoneal injection of the virus.

In experiments 1 and 2, the inoculation dose was extremely high ~~with the result~~ that the virulence of the organs progressively decreased.

Virus was found in the spleen and liver for longer periods than in the circulating blood. In experiment 3, virus was injected in lower doses. The results are presented in Fig. 2.

Fig. 2. Multiplication of the neurotropic virus of RVP and appearance of neutralizing antibodies in the blood after intraperitoneal inoculation of mice

- 1 - LD<sub>50</sub> or neutralization index in log
- 2 - Inoculation dose
- 3 - Interval after inoculation (in days)
- 4 - Spleen
- 5 - Liver
- 6 - Blood
- 7 - Antibody

Virus could not be detected in any of the organs examined after 5 hours. After 24 hours it was found in the spleen only in the spleen. On day 2 it appeared in the spleen and liver, but not in the circulating blood. The virulence of the spleen and liver reached a peak on day 3 after which it slowly decreased. The circulating blood ~~was~~ became virulent on day 3. Its virulence decreased fairly quickly.

The neutralizing antibodies appeared in the circulating blood on days 3 to 5 after injection of the virus. Its titer rose progressively (Figs. 1 and 2). It will be noted that the virus could still be detected even after the neutralizing antibodies appeared.

Serial passage of the neurotropic strain in the mouse spleen.  
We inoculated mice intraperitoneally with 0.2 ml of a 10% emulsion of infected brains. After 4 days the mice were sacrificed and their livers removed to make a 10% emulsion. Mice were injected intraperitoneally with 0.5 ml. This is the way the passage was carried out.

Serial passage was accomplished easily. The virulence of

the spleen was determined in each generation by intracerebral injection. The symptoms provoked by peritoneal injection did not differ from those observed after intracerebral injection of the original strain.

The 14th generation virus was intracerebrally injected.

~~when nervous symptoms appeared~~, the animals were sacrificed. The virulence of the brain suspension was

determined by intracerebral injection of part of it and by subcutaneous injection of another part. After subcutaneous

injection the LD<sub>50</sub> was 10 or less. After intracerebral injection it was 10. The virus was neutralized by

rabbit serum against the neurotropic strain when ~~the mice were~~ injected intraperitoneally with a mixture of virus and serum.

#### Summary

(1) ~~Rift Valley fever~~ The neurotropic strain of virus multiplied in the

spleen and liver of mice injected with it intraperitoneally.

(ii) Virus was detected in the spleen 41 days after injection.

(iii) Virus was detected for several days in the circulating blood even after the appearance of neutralizing antibodies.

(iv) After serial passage ~~of~~ in the mouse liver, very low doses of virus injected intraperitoneally killed mice with nervous symptoms.

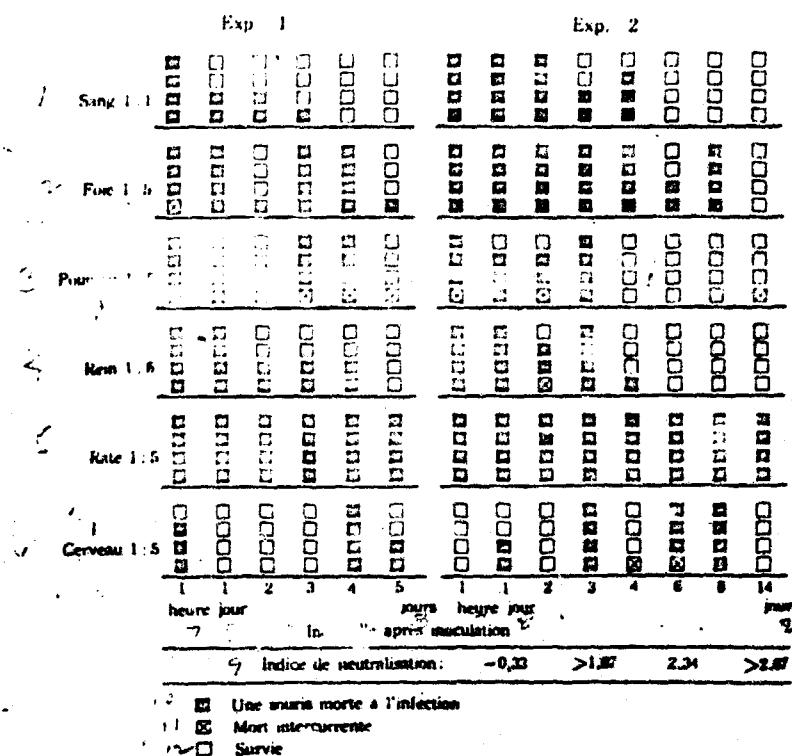


Fig. 1. — Distribution du virus neurotrophe de la FVR et le pouvoir neutralisant du sang après l'inoculation de la souris par voie péritonale.

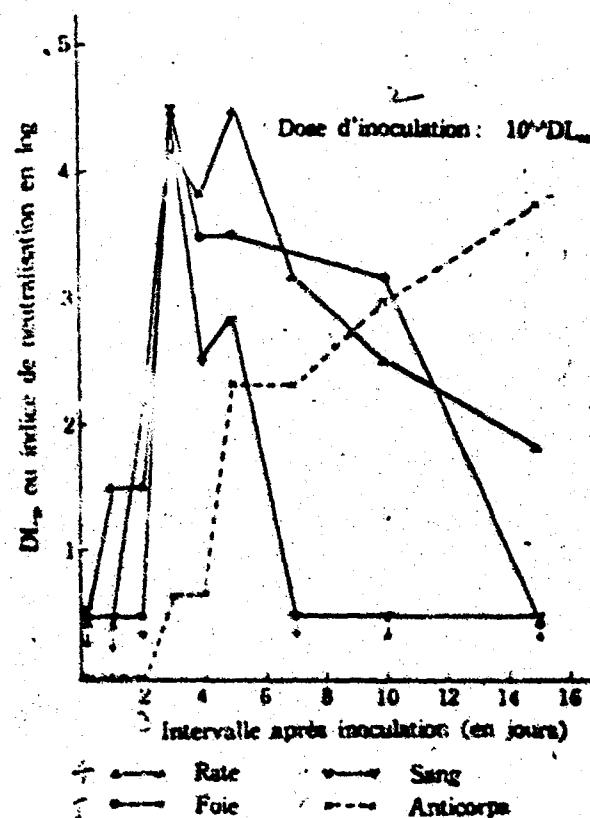


Fig. 2. — Multiplication du virus neurotrophe de la FVR et apparition d'anticorps neutralisants dans le sang après l'inoculation de la souris par voie intraperitoneale.